Practical Aspects of Membrane Protein Crystallography: From Overexpression to Crystallization Workshop

May 20, 2002

Integral membrane proteins perform some of the most important functions of living cells, yet understanding their molecular mechanisms through structural studies presents unique challenges. The aim of the workshop entitled "Practical Aspects of Membrane Protein Crystallography: From Overexpression to Crystallization" was to discuss the nuts-and-bolts of over-expressing, purifying, and crystallizing membrane proteins for structural studies by x-ray crystallography.

Biologist Susan Buchanan, of the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) at the National Institute of Health (NIH), made a presentation on the production, purification, and characterization of bacterial outer membrane pro-

teins both refolded from inclusion bodies and also as membrane-inserted functional proteins.

Cell biologist Da Neng Wang, of New York University, described the production of bacterial inner membrane transporters as functional, affinity-tagged proteins, and methods for optimization of each step in their production and purification.

Biologist Martine Cadene, of Rockefeller University in New York, explained the use of the Matrix Assisted Laser Desorption Ionization (MALDI) mass spectrometry in defining the covalent state of purified membrane proteins, and using this information as an aid in designing mutant constructs specifically amenable to crystallographic analysis.

Biologist Miro Venturi, of NIH, described methods for the production and screening of monoclonal antibodies specifically reactive against the folded conformations of integral membrane proteins. He described recombinant methods for production and purification of Fab and Fv fragments of these antibodies, and their use as crystallization reagents, giving work with the transporter protein NhaA as a specific example.

Structural biologist Reinhard Grisshammer, of NIH, "Expression and Purification of G-Protein-Coupled Receptors (GPCRs) for Structure Determination", gave a detailed presentation on the expression and purification of functional G-protein coupled receptors as fusion proteins in E. coli. He gave examples of success with this approach for several different GPCRs.

Barry Springer, director of Molecular Biology and Protein Biochemistry and project team leader at 3-Dimensional Pharmaceuticals Inc. (3DP), presented the expression of functional GPCRs in mammalian (HEK-293) cells, and biophysical and fluorimetric high-throughput assays to assess their functionality and stability in detergent-containing solutions.

Biophysicist Ehud Landau, University of Texas Medical Branch in Galveston, described the use of cubic lipidic matrices as a medium for the stable incorporation of integral membrane proteins, and their crystallization in this medium. He presented many examples of highly diffracting crystals obtained using this methodology.

BNL biologist Dax Fu described the stabilization of solubilized bacterial transporters by inclusion of their cognate binding ligands, a necessary precursor to their successful crystallization.

Biochemist Patrick Loll, of Drexel University in Philadelphia, presented a rational approach to the design of crystallization screens for membrane proteins, based on the "cloud points" of detergents in different protein-precipitating conditions. Based on this approach, he has developed screens containing over 600 potentially useful conditions, near detergent phase transitions, and demonstrated a high rate of success with this screen.

-Larry Shapiro and Filippo Mancia

Workshop Participants